

**IN THE CLAIMS:**

1. (original) A method for characterizing proteins comprising:
  - a) providing:
    - i) a sample comprising a plurality of proteins;
    - ii) a first separating apparatus that separates proteins based on a first physical property;
    - iii) a second separating apparatus that separates proteins based on a second physical property; and
    - iv) a mass spectrometry apparatus;
  - b) treating said sample with said first separating apparatus to produce a first separated protein sample;
  - c) treating at least a portion of said first separated protein sample with said second separating apparatus to produce a second separated protein sample; and
  - d) directly feeding said second separated protein sample from said second separating apparatus to said mass spectrometry apparatus; and
  - e) mass spectrally analyzing at least a portion of said second separated protein sample with said mass spectrometry apparatus to characterize protein mass.
2. (original) The method of Claim 1, wherein said sample comprises a cell lysate.
3. (original) The method of Claim 1, wherein said first physical property is protein charge.
4. (original) The method of Claim 1, wherein said first separating apparatus comprises an isoelectric focusing apparatus.
5. (original) The method of Claim 1, wherein said first separating apparatus comprises a liquid phase separating apparatus.

6. (original) The method of Claim 1, wherein said second separating apparatus comprises a reverse phase HPLC apparatus.

7. (original) The method of Claim 6, wherein said reverse phase HPLC comprises non-porous reverse phase HPLC.

8. (original) The method of Claim 1, wherein said mass spectrometry apparatus comprises an ESI or TOF mass spectrometry apparatus.

9. (original) The method of Claim 1, further comprising the step of d) displaying at least said first physical property of at least a portion of said second separated protein sample.

10. (original) The method of Claim 9, wherein said displaying comprises a schematic representation of first and second physical properties of at least a portion of said second separated protein sample.

11. (original) The method of Claim 10, wherein said first and second properties comprise pI and hydrophobicity.

12. (original) The method of Claim 10, wherein said schematic representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

13. (original) The method of Claim 10, wherein said schematic representation further displays protein abundance of proteins represented in said schematic representation.

14. (original) The method of Claim 13, wherein proteins are represented as bands in said schematic representation.

15. (original) The method of Claim 14, wherein protein abundance correlates to intensity of said bands.

16. (original) The method of Claim 14, wherein said schematic representation has a resolution that allows the differentiation of a first band representing a first protein and a second band representing a phosphorylated version of said first protein.

17. (original) The method of Claim 1, wherein said sample comprising a plurality of proteins further comprises a buffer, wherein said plurality of proteins are solubilized in said buffer and wherein said buffer is compatible with said first and said second separating apparatus.

18. (original) The method of Claim 17, wherein said buffer is further compatible with said mass spectrometry apparatus.

19. (original) The method of Claim 17, wherein said buffer comprises a compound of the formula n-octyl C6-C12 glycopyranoside.

20. (original) The method of Claim 19, wherein said compound of the formula n-octyl C6-C12 glycopyranoside is selected from n-octyl  $\beta$ -D-glucopyranoside and n-octyl  $\beta$ -D-galactopyranoside.

21. (original) A system comprising:

- a) a first separating apparatus that separates proteins based on a first physical property; and
- b) a non-porous reverse phase HPLC apparatus directly connected to a mass spectrometry apparatus, wherein proteins separated by said non-porous reverse phase HPLC apparatus are directly fed to said mass spectrometry apparatus without intervening processing.

22. (original) The system of Claim 21, wherein said first separating apparatus comprises a liquid phase separating apparatus.

22~~3~~. (presently amended) The system of Claim 22, wherein said liquid phase first separating apparatus comprises an isoelectric focusing apparatus.

23~~4~~. (presently amended) The system of Claim 21, wherein said mass spectrometry apparatus comprises an ESI or TOF mass spectrometry apparatus.

24~~5~~. (presently amended) The system of Claim 21, further comprising a detector that detects proteins separated by said second separating apparatus.

25~~6~~. (presently amended) The system of Claim 24~~5~~, further comprising a processor configured to run protein display software, wherein said protein display software produces a data representation of detected proteins.

26~~7~~. (presently amended) The system of Claim 25~~6~~, further comprising a display that displays said data representation, wherein said first physical property, said second physical properties, and protein abundance of at least a portion of said plurality of proteins are represented.

27~~8~~. (presently amended) The system of Claim 26~~7~~, wherein said first and second properties comprise pI and hydrophobicity.

28~~9~~. (presently amended) The system of Claim 26~~7~~, wherein said data representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

2930. (presently amended) The system of Claim 267, wherein proteins are represented as bands in said data representation.

301. (presently amended) The system of Claim 2930, wherein protein abundance correlates to intensity of said bands.

342. (presently amended) The system of Claim 2930, wherein said data representation has a resolution that allows the differentiation of a first band representing a first protein and a second band representing a phosphorylated version of said first protein.

323. (presently amended) A method for characterizing proteins comprising:

- a) providing:
  - i) a sample comprising a plurality of proteins;
  - ii) a first separating apparatus;
  - iii) a non-porous reverse phase HPLC apparatus; and
  - iv) a mass spectrometry apparatus;
- b) treating said sample with said first separating apparatus to produce a first separated protein sample, wherein said first separated protein sample is collected from said first separating apparatus in a plurality of fractions, each of said fractions defined by a distinct pH range;
- c) treating at least a portion of said first separated protein sample from at least one of said fractions with said non-porous reverse phase HPLC apparatus to produce a second separated protein sample; and
- d) mass spectrally analyzing at least a portion of said second separated protein sample with said mass spectrometry apparatus to characterize protein mass.

334. (presently amended) The method of Claim 323, wherein said second separated protein sample is directly fed to said mass spectrometry apparatus without an intervening protein digestion step.

~~345~~. (presently amended)      The method of Claim ~~323~~, wherein said sample comprises a cell lysate.

~~356~~. (presently amended)      The method of Claim ~~323~~, wherein said first separating apparatus separates proteins base on charge.

~~367~~. (presently amended)      The method of Claim ~~356~~, wherein said first separating apparatus comprises an isoelectric focusing apparatus.

~~378~~. (presently amended)      The method of Claim ~~323~~, wherein said first separating apparatus comprises a liquid phase separating apparatus.

~~389~~. (presently amended)      The method of Claim ~~323~~, wherein said mass spectrometry apparatus comprises an ESI oa TOF mass spectrometry apparatus.

~~3940~~. (presently amended)      The method of Claim ~~323~~, further comprising the step of e) displaying at least a first physical property of at least a portion of said second separated protein sample.

~~401~~. (presently amended)      The method of Claim ~~3940~~, wherein said displaying comprises a schematic representation of first and second physical properties of at least a portion of said second separated protein sample.

~~412~~. (presently amended)      The method of Claim ~~401~~, wherein said first and second properties comprise pI and hydrophobicity.

~~423~~. (presently amended)      The method of Claim ~~401~~, wherein said schematic representation comprises a 2-dimensional protein map, wherein said first physical property is

represented on a first axis and wherein said second physical property is represented on a second axis.

434. (presently amended) The method of Claim 401, wherein said schematic representation further displays protein abundance of proteins represented in said schematic representation.

445. (presently amended) The method of Claim 323, wherein said sample comprising a plurality of proteins further comprises a buffer, wherein said plurality of proteins are solubilized in said buffer and wherein said buffer is compatible with said first and said second separating apparatus.

456. (presently amended) The method of Claim 445, wherein said buffer is further compatible with said mass spectrometry apparatus.

467. (presently amended) The method of Claim 445, wherein said buffer comprises a compound of the formula n-octyl C6-C12 glycopyranoside.

478. (presently amended) The method of Claim 467, wherein said compound of the formula n-octyl C6-C12 glycopyranoside is selected from n-octyl  $\beta$ -D-glucopyranoside and n-octyl  $\beta$ -D-galactopyranoside.

489. (presently amended) A system comprising:

- a) a first separating apparatus;
- b) a buffer that elutes protein from said first separating apparatus in a plurality of fractions, each of said fractions defined by a distinct pH range;
- c) a first delivery apparatus capable of receiving separated protein in said plurality of fractions;

d) a non-porous reverse phase HPLC apparatus configured to receive proteins from said first delivery apparatus; and

e) a mass spectrometry apparatus configured to receive proteins from said non-porous reverse phase HPLC apparatus.

4950. (presently amended) The system of Claim 489, wherein said mass spectrometry apparatus is configured to directly receive proteins from said non-porous reverse phase HPLC apparatus.

501. (presently amended) The system of Claim 489, wherein said first separating apparatus comprises a liquid phase separating apparatus.

512. (presently amended) The system of Claim 501, wherein said liquid phase first separating apparatus comprises an isoelectric focusing apparatus.

523. (presently amended) The system of Claim 489, wherein said mass spectrometry apparatus comprises an ESI or TOF mass spectrometry apparatus.

534. (presently amended) The system of Claim 489, further comprising a detector that detects proteins separated by said non-porous reverse phase HPLC apparatus.

545. (presently amended) The system of Claim 534, further comprising a processor configured to run protein display software, wherein said protein display software produces a data representation of detected proteins.

556. (presently amended) The system of Claim 545, further comprising a display that displays said data representation, wherein a first physical property, a second physical properties, and protein abundance of at least a portion of said plurality of proteins are represented.



***PATENT***

Attorney Docket No. **UM-6102**

**567.** (presently amended) The system of Claim **556**, wherein said first and second properties comprise pI and hydrophobicity.

**578.** (presently amended) The system of Claim **556**, wherein said data representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

**589.** (presently amended) An automated protein characterization system comprising:

- a) a first separating apparatus;
- b) an automated sample handling device;
- c) a second separating apparatus operably linked to said first separating apparatus and said sample handling device, wherein said second separating apparatus is configured to receive proteins from said first separating apparatus;
- d) a mass spectrometry apparatus operably linked to said second separating apparatus and said sample handling device; wherein said mass spectroscopy apparatus is configured to receive proteins from said second separating apparatus; and
- e) a processor that controls said sample handling device, said first separating apparatus, said second separating apparatus; and said mass spectrometry apparatus.

**5960.** (presently amended) The system of Claim **589**, wherein said processor comprises computer memory and a computer processor.

**601.** (presently amended) The system of Claim **589**, wherein said processor is configured to produce a data representation of separated proteins analyzed by said mass spectrometry apparatus.

**612.** (presently amended) The system of Claim **589**, wherein said automated sample handling device comprises a switchable, multi-channel valve.

***PATENT***

Attorney Docket No. **UM-6102**

623. (presently amended) The system of Claim 601, further comprising a display that displays said data representation.

634. (presently amended) The system of Claim 601, wherein said data representation comprises a first dimension representing protein charge.

645. (presently amended) The system of Claim 634, wherein said representation further comprises a second dimension representing protein molecular weight.

656. (presently amended) The system of Claim 654, wherein said data representation further represents protein abundance.

667. (presently amended) The system of Claim 589, wherein said first separating apparatus comprises a liquid phase separating apparatus.

678. (presently amended) The system of Claim 667, wherein said liquid phase first separating apparatus comprises an isoelectric focusing apparatus.

689. (presently amended) The system of Claim 589, wherein said second separating apparatus comprises a non-porous reverse phase HPLC apparatus.

6970. (presently amended) The system of Claim 589, wherein said mass spectrometry apparatus comprises an ESI or TOF mass spectrometry apparatus.

701. (presently amended) The system of Claim 689, wherein said mass spectrometry apparatus is configured to directly receive proteins from said non-porous reverse phase HPLC apparatus.

712. (presently amended) The system of Claim 589, further comprising a solid phase extraction apparatus configured to treat proteins separated by said first separating apparatus prior to delivery of proteins to said second separating apparatus.

723. (presently amended) An automated method for separating proteins comprising:

- a) providing:
  - i) a sample comprising a plurality of proteins,
  - ii) a first separating apparatus that separates proteins based on a first physical property;
  - iii) a second separating apparatus that separates proteins based on a second physical property;
  - iv) a mass spectroscopy apparatus; and
  - v) an automated sample handling device comprising a switchable, multi-channel valve;
- b) treating said sample with said first separating apparatus to produce a first separated protein sample, wherein said first separated protein sample is collected from said first separating apparatus in a plurality of fractions, each of said fractions defined by a distinct range of said first physical property;
- c) transferring said first separated protein sample to said second separating apparatus using said automated sample handling device;
- d) treating said first separated protein sample with said second separating apparatus to produce a second separated protein sample;
- e) transferring said second separated protein sample to said mass spectroscopy apparatus using said automated sample handling device; and
- f) mass spectrally analyzing said second separated protein sample with said mass spectroscopy apparatus to characterize protein mass.

734. (presently amended) The method of Claim 723, further comprising a centralized control network operably linked to said automated sample handling device, said first separating

***PATENT***

Attorney Docket No. **UM-6102**

apparatus, said second separating apparatus, and said mass spectroscopy apparatus, wherein said centralized control network is configured to control said automated sample handling device, said first separating apparatus, said second separating apparatus, and said mass spectroscopy apparatus.

745. (presently amended) The method of Claim 723, further comprising providing a solid phase extraction apparatus, wherein prior to treating said first separated sample with said second apparatus; said first separated sample is treated with said solid phase extraction apparatus.

756. (presently amended) The method of Claim 723, wherein said sample comprises a cell lysate.

767. (presently amended) The method of Claim 723, wherein said first physical property is protein charge.

778. (presently amended) The method of Claim 723, wherein said first separating apparatus comprises an isoelectric focusing apparatus.

789. (presently amended) The method of Claim 723, wherein said first separating apparatus comprises a liquid phase separating apparatus.

7980. (presently amended) The method of Claim 723, wherein said second separating apparatus comprises a reverse phase HPLC apparatus.

801. (presently amended) The method of Claim 7980, wherein said reverse phase HPLC comprises non-porous reverse phase HPLC.

***PATENT***

Attorney Docket No. **UM-6102**

812. (presently amended) The method of Claim 723, wherein said mass spectrometry apparatus comprises an ESI or TOF mass spectrometry apparatus.

823. (presently amended) The method of Claim 723, further comprising the step of g) displaying at least said first physical property of at least a portion of said second separated protein sample.

834. (presently amended) The method of Claim 823, wherein said displaying comprises a schematic representation of first and second physical properties of at least a portion of said second separated protein sample.

845. (presently amended) The method of Claim 834, wherein said first and second properties comprise pI and hydrophobicity.

856. (presently amended) The method of Claim 834, wherein said schematic representation comprises a 2-dimensional protein map, wherein said first physical property is represented on a first axis and wherein said second physical property is represented on a second axis.

867. (presently amended) The method of Claim 834, wherein said schematic representation further displays protein abundance of proteins represented in said schematic representation.

878. (presently amended) The method of Claim 723, wherein said sample comprising a plurality of proteins further comprises a buffer, wherein said plurality of proteins are solubilized in said buffer and wherein said buffer is compatible with said first and said second separating apparatus.

***PATENT***

Attorney Docket No. **UM-6102**

889. (presently amended) The method of Claim 878, wherein said buffer is further compatible with said mass spectrometry apparatus.

8990. (presently amended) The method of Claim 878, wherein said buffer comprises a compound of the formula n-octyl C6-C12 glycopyranoside.

901. (presently amended) The method of Claim 8990, wherein said compound of the formula n-octyl C6-C12 glycopyranoside is selected from n-octyl  $\beta$ -D-glucopyranoside and n-octyl  $\beta$ -D-galactopyranoside.